MatTek Corporation

May 15, 2009

MatTek Corporation response to Federal Register Notice (Vol. 74(60):14556, 2009): Request for public comment on the background review document (BRD), draft ICCVAM summary review document (SRD), and draft ICCVAM recommendations on an In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products.

We are pleased that the EpiOcular model was chosen as one of the test systems for development of ocular hazard assessment assays as described in the Background Review Document (BRD) of an In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products. We also very much appreciate the efforts expended by the authors and the Alternative Testing Working Group to conduct the studies and prepare the documents. However, after careful review of the data presented in the BRD, we have concerns that this document significantly understates the true potential utility of the EpiOcular assay in comparison to the BCOP assay for this important application. Specific detailed comments are presented below. We request that the ICCVAM and the Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods consider these comments and incorporate them into the Final ICCVAM SRD and recommendations on this topic.

1. Removal of Restriction on Testing of Oxidant Chemicals in the EpiOcular Assay.

The BRD proposes a scheme for testing of anti-microbial cleaning products in which products containing oxidant chemicals are automatically excluded from testing in the EpiOcular assay (BRD, p xxiv). The rationale given for this decision is that EpiOcular test results did not match well (i.e. EpiOcular predicted more severe irritation) compared to in vivo data obtained in the Low Volume Eye Test (LVET) (BRD, Section 6, p108). The same comparison of BCOP data to LVET data was not made.

When compared to in vivo Draize data, EpiOcular test results with oxidant chemicals were in 100% agreement (BRD, Section 6, p116). In contrast, oxidant chemicals are known to be problematic in the BCOP test, even in comparison to Draize data. Oxidants are often under-predicted by the BCOP assay, and require histological assessment for correct prediction (BRD, Section 6, p135). Furthermore, BCOP data for oxidant chemicals showed only 62% correct predictions when compared to Draize data, with 19% over-prediction and 19% under-prediction (BRD, Section 6, p128). Additionally, as noted in the Draft Proposed ICCVAM Test Method Recommendations: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches (April 1, 2009) (Section 2.0, p11):

- LVET under-predicts severe irritants compared to the Draize.
- There are insufficient data to evaluate the extent of (LVET) under-prediction relative to known human severe ocular irritants.
- There is an inconsistent relationship between LVET and Draize results (i.e., time-to-clear) for substances with available human data.
- Accordingly, ICCVAM proposes that the LVET has not been adequately validated and does not have adequate demonstrated performance (sensitivity and specificity) to serve as an acceptable reference test method against which to determine the validity of *in vitro* alternative test methods for hazard classification and labeling purposes.

200 Homer Avenue **(508) 881-6771**
Ashland MA 01721 ***Web Site:*** **www.mattek.com** ***Email:*** **information@mattek.com** **Fax (508) 879-1532**

Thus, the rationale presented in the BRD for excluding EpiOcular for use with oxidant chemicals is flawed and this restriction on the EpiOcular assay should be removed. In making their draft recommendations, ICCVAM considered EpiOcular data presented for all chemicals including oxidants in their determination of the usefulness the EpiOcular assay. However, they did not explicitly comment on the recommendation presented in the BRD to automatically exclude oxidants from being tested in the EpiOcular assay. Based on the data presented in the BRD in comparison to in vivo Draize data, we ask ICCVAM and the Independent Peer Review Panel to explicitly comment on the usefulness of the EpiOcular Assay with oxidant chemicals and the unwarranted recommendation presented in the BRD to exclude the EpiOcular assay from use in testing oxidant chemicals.

2. Correction and Clarification of Criteria for Use of the EpiOcular Assay and the Cytosensor Assay.

The ICCVAM Draft Summary Review Document (SRD): Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products Using *In Vitro* Alternative Test Methods (SRD) (April 1, 2009) misstates the scheme proposed in the BRD (p 6, Figure 1-1) with regard to the appropriate criteria for use of the EpiOcular assay and the Cytosensor assay. The SRD (p xxi) incorrectly interprets the BRD scheme as stating that, "Selection of the CM (Cytosensor Microphysiometer) or EO (EpiOcular) depends on water solubility of the test substance; water-soluble substances would be tested in the CM and water-insoluble substances would be tested in the EO to determine the final hazard classification." The scheme actually indicates that water soluble test agents can be tested in either assay (BRD p122), but water insoluble test agents are incompatible with the Cytosensor assay, and therefore can only be tested in the EpiOcular assay. Although this may be a moot point given that Cytosensor instrumentation and related supplies are no longer be available, we ask that this statement be corrected in the SRD and clarified in the BRD.

3. Removal of Restriction on Use of the EpiOcular Assay for Determination of Category I (Cat I) Chemicals.

The BRD implies that the BCOP assay is most useful for the severe categories and that EpiOcular should only be used for the milder categories (BRD p xxxi). Papers published by Stern et al. (Toxicology In Vitro, 12, 455-461, (1998)) and Jones et al (ATLA 29, 669-692, (2001)) are cited with regard to assertions that the EpiOcular assay functions particularly well at the mild end of the ocular irritation spectrum (BRD p121). While this is indeed true, this should NOT, however, be interpreted as indicating that the EpiOcular assay does not function well at the severe end of the ocular irritation spectrum as well. In fact, the Jones et al paper states that, "The EpiOcular assay showed the closest concordance between the in vivo results and the in vitro data from cell-based assays..." Likewise, the Stern at al paper found an overall high concordance between in vivo Draize data and in vitro EpiOcular data. In contrast, Jones et al also found that, "The BCOP assay was less sensitive than the IRE test in discriminating between formulations of different irritation potentials, and did not perform as well as the other assays in identifying mild formulations."

The BRD also states that the EpiOcular assay ***cannot*** distinguish between Cat I and Cat II chemicals (BRD p xxxi), and that the BCOP ***can*** effectively distinguish between EPA Cat I and II chemicals (BRD xxxi). The data presented in the BRD are inconsistent with these claims as well.

A summary of the BCOP and EpiOcular data are complied for reference in Tables I and II below. The data summarized in Table I show that the performance of the EpiOcular assay was superior to that of the BCOP assay at both ends of the ocular irritation spectrum. The EpiOcular assay produced 100% sensitivity and 88% predictivity for Cat I chemicals, while for the BCOP assay, sensitivity ranged from 84-92% and predictivity ranged from 77-87%. Thus, while both the BCOP and EpiOcular assays appear to be useful for determining Cat I chemicals, the EpiOcular assay clearly performed better than the BCOP. Therefore, exclusion of the EpiOcular assay for determination of Cat 1 chemicals is not justified by the data and this restriction on the EpiOcular assay should be removed from the finalized testing strategy proposed in the BRD (p xxiv).

Regarding Cat II chemicals, EpiOcular was only tested with 1 chemical, which it underpredicted as a Cat III. Therefore, while additional testing of Cat II materials in the EpiOcular assay is warranted, the currently available data do not provide any basis for stating that the EpiOcular test cannot distinguish between Cat I and Cat II chemicals. For Cat II chemicals, the BCOP assay provided only very low sensitivity (ranging from 20-60%) and predictivity (ranging from 17-38%). Furthermore, following the procedure recommended for the BCOP in the BRD, for chemicals testing preliminarily as Cat II, "they should be further assessed with a histopathological evaluation and given the final categorization of whichever determination (in vitro score or histological evaluation) is more severe," (BRD, p145). This procedure is expected to overpredict 80% of Cat II chemicals as Cat I (BRD, Table 6-50, p141). Thus, the BCOP cannot be regarded as a useful assay for predicting Cat II chemicals or distinguishing between Cat I and Cat II chemicals.

Data presented in the BRD do show, however, that the high solvent (HS) BCOP assay plus histology is effective for distinguishing between Cat I plus II and Cat III (BRD Table 6-50, p141). Thus, after first removing true Cat I chemicals in preliminary tests (e.g with the EpiOcular assay), the BCOP plus histology assay (if fully developed and approved) may be useful for distinguishing between Cat II and Cat III (see new testing strategy proposed in section 4 below).

4. Proposal for Improved Testing Strategy for Use of the EpiOcular Assay and the BCOP Assay for Determination of EPA Hazard Classification of Anti-Microbial Cleaning Products.

With unwarranted restrictions on testing of oxidant chemicals and use for determination of Cat I chemicals removed from the EpiOcular assay, the following testing strategy for determination of EPA hazard classification of anti-microbial cleaning products is most consistent with the data presented in the BRD. This strategy will represent the best solution for the EPA in terms of accuracy, time required for assay performance, cost and ease of use (Figure 1) (see also BRD p122 for similar scheme). According to the proposed strategy, chemicals should be tested first in the EpiOcular assay to determine Cat I, Cat II plus III and Cat IV classifications. These 3 classifications will provide the most important information (i.e. irreversible, reversible or minimal eye irritation potential). If Cat I or Cat IV are determined, no further testing is required. For chemicals testing as Cat II plus III, if a broad classification of reversible is acceptable to the manufacturer, no further testing is required. However, if a distinction between Cat II (reversible within 21 days) and Cat III (reversible within 7 days) is desired, further testing with the BCOP (with solvent concentration accounted for and histology assessment, when fully developed) can be performed to determine Cat II or Cat III (with this assay protocol, no Cat II chemicals are underpredicted as Cat III, BRD, Table 6-50, P141). Further testing and refinement of the EpiOcular assay may also ultimately allow separation of Cat II and Cat III chemicals.

The ICCVAM draft SRD conducted an analysis of the 28 chemicals with available Draize data that were tested in common between the EpiOcular Assay and the BCOP assay (SRD Table 2, p xxx, and Appendix G). Two approaches utilizing BCOP for testing Cat I chemicals and EpiOcular for testing Cat IV chemicals were evaluated. One approach involved testing of all chemicals in the BCOP first, removing chemicals determined to be Cat I, and re-testing the remaining chemicals in the EpiOcular assay to determine Cat IV chemicals. The alternate approach tested the chemicals in the EpiOcular assay first, and after removal of all Cat IV chemicals, re-testing of the remaining chemicals in the BCOP assay to determine Cat I chemicals. Both approaches correctly categorized 79% of the test chemicals, and were described as useful for determining Cat I and Cat IV chemicals in the ICCVAM draft recommendations.

However, analysis of data presented in SRD Appendix G show that when the BCOP assay was performed first, only 64% of all chemicals including 100% of Cat I, but 0% of Cat IV chemicals were correctly categorized by the BCOP assay. However, if the EpiOcular assay is performed first, the 79% concordance is immediately obtained, including 100% Cat I, 100% of Cat III and 44% of Cat IV chemicals with no under-prediction of the more severe categories. Conducting the BCOP assay after the EpiOcular assay would thus unnecessarily waste time and resources without any added benefit.

Therefore, based on the data presented in the SRD, Appendix G, we ask ICCVAM and the Independent Peer Review Panel to accord the same degree of "usefulness" to the EpiOcular assay as a stand-alone assay as is accorded to the tiered assays combining BCOP and EpiOcular in the Final SRD and Recommendation documents.

5. Summary and Final Comments

Based on the detailed comments presented above, we request that the ICCVAM and the Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods consider the following items and incorporate specific responses to them in the Final ICCVAM SRD and Recommendations on this topic.

- Removal of the restriction on testing of oxidants in the EpiOcular assay.
- Correction and clarification of criteria for use of the EpiOcular Assay and the Cytosensor Assay.
- Removal of the restriction on use of the EpiOcular Assay for determination of Cat I chemicals.
- Proposal for improved testing strategy for use of the EpiOcular Assay and the BCOP Assay for determination of EPA hazard classification of anti-microbial cleaning products.
- Explicit acknowledgment that the EpiOcular assay provided overall superior performance compared to the BCOP assay for the common chemical set tested and compared against Draize data. Furthermore, acknowledgement that combining the BCOP and EpiOcular assay did not provide any benefit to results obtained by the EpiOcular assay alone.
- In addition to the ICCVAM finding that a combination of BCOP and EO appear to be useful for determination of Cat I and Cat IV, the EpiOcular assay has the identical utility for determining these categories by itself as a stand alone method. The BCOP assay in contrast is only useful for determining Cat I as a stand alone assay.

MatTek Corporation values and appreciates its close working relationship with the institutions and companies involved in development of animal alternative methods such as the EPA hazard classification methods proposed here. We look forward to continuing this close cooperation in order to develop any new EpiOcular data that may be required to support full validation of the EpiOcular in vitro test method for determination of EPA Toxicity Labeling of Anti-Microbial Cleaning Products. However, it is

important that the concerns raised here are adequately addressed in order to insure that unjustified restrictions are not imposed on the EpiOcular Assay during future studies.

Table I. Summary of BCOP and EpiOcular performance compared to Draize data presented in the BRD of an In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products

	BCOP			EpiOcular	
	Assay Sensitivity (Assay Predictivity) (%)[6]				
	Std Protocol[1]	HS Protocol/WO Hist[2]	HS Protocol/W Hist[3]	W Oxidiants[4]	WO Oxidants[5]
Cat I	90 (87)	84 (84)	92 (77)	100 (88)	100 (86)
Cat II	60 (27)	60 (38)	20 (17)	0 (0)[7]	0 (0)[7]
Cat III	50 (25)	58 (25)	58 (/28)	75 (38)	75 (38)
Cat IV	0 (0)	0 (0)	0 (0)	44 (100)	38 (100)
Overall Correct Classification	54.5 %	49 %	51 %	76 %	72 %

[1]Standard BCOP protocol: BRD Table 6-40, P128.
[2]High solvent BCOP protocol without histology: BRD Table 6-44, P133.
[3]High solvent BCOP protocol with histology: BRD Table 6-50, P141.
[4]EpiOcular data compared to Draize data including oxidant chemicals: BRD Table 6-29, P116.
[5]EpiOcular data compared to Draize data not including oxidant chemicals: BRD Table 6-31, P118.
[6]Sensitivity is defined as the proportion of true positives that are correctly identified by the test and predictivity is defined as the proportion of total positive predictions that are correct.
[7]Based on only 1 Cat II chemical tested.

Table II. Summary of BCOP and EpiOcular performance compared to Draize data presented in the BRD of an In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products: Cat I vs. Cat II-III-IV.

	BCOP		EpiOcular	
	Assay Sensitivity[1]			
	Std Protocol[2]	HS Protocol/WO Hist[3]	HS Protocol/W Hist[4]	EpiOcular[5]
Cat I	27/30 (90%)	21/25 (84%)	23/25 (92%)	15/15 (100%)
Cat II-III-IV	30/36 (83%)	32/36 (88.9%)	29/36 (80.5%)	12/14 (85.7)

[1]Sensitivity is defined as the proportion of true positives that are correctly identified by the test
[2]Standard BCOP protocol: BRD Table 6-40, P128.
[3]High solvent BCOP protocol without histology: BRD Table 6-44, P133.
[4]High solvent BCOP protocol with histology: BRD Table 6-50, P141.
[5]EpiOcular data compared to Draize data including oxidant chemicals: BRD Table 6-29, P116.

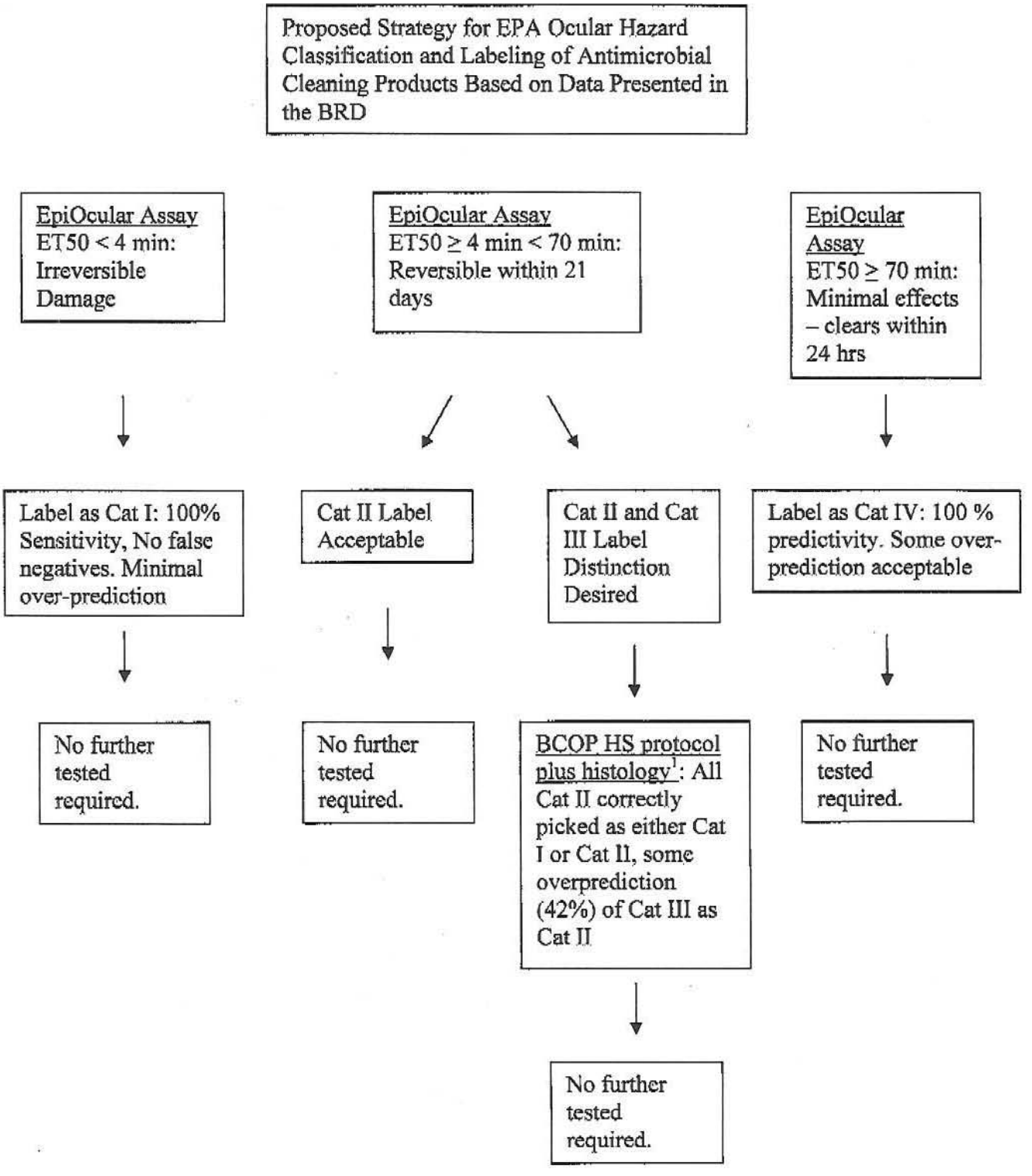

Figure 1. Proposed Strategy for EPA Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products Based on Data Presented in the BRD. [1]High solvent BCOP protocol with histology: BRD Table 6-50, P141.